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ORIGINAL ARTICLE

Serum eosinophilic cationic protein and high sensitive C-reactive protein as alternative parameters for differentiation of severity stages and monitoring control in bronchial asthma patients

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Abstract *Background:* High sensitive C-reactive protein (hs-CRP) and eosinophilic cationic protein (ECP) have been shown to be associated with asthma in recent studies. However, the relationship between hs-CRP and the control of asthma has not been clearly identified yet.

Patients and methods: Thirty patients with newly-diagnosed bronchial asthma and 20 healthy individuals were enrolled in this study. In addition to medical history and physical examination, asthma was assessed according to the Global Initiative for Asthma (GINA) guidelines. Respiratory function tests (RFT), serum hs-CRP and ECP levels, serum total IgE levels, circulating eosinophil count (CE) and asthma control test (ACT) were performed for all subjects.

Results: Serum ECP, serum hs-CRP, serum total IgE and CE were significantly higher ($p < 0.01$, 0.01, 0.05 and 0.05, respectively), while forced expiratory volume in 1 s (FEV_1 %) and ACT were significantly lower ($p < 0.05$) in asthmatic patients compared to the control group. In all patients with bronchial asthma, serum levels of hs-CRP and ECP showed significant positive correlations with asthma severity (hs-CRP, $r_s = 0.59$, $p < 0.01$; ECP, $r_s = 0.63$, $p < 0.01$, respectively) but, significant negative correlations with ACT (hs-CRP, $r_s = -0.53$, $p < 0.05$; ECP, $r_s = -0.62$, $p < 0.01$, respectively) and FEV_1 % (hs-CRP, $r_s = -0.46$, $p < 0.05$; ECP, $r_s = -0.57$, $p < 0.01$, respectively). Serum ECP and hs-CRP levels showed significant fall ($p < 0.01$and $p < 0.05$, respectively), while, FEV_1 % and ACT showed significant increase ($p < 0.05$) in asthmatic patients who were followed up after 2 months of therapy.

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Conclusion: Coupling measurements of serum levels of both ECP and hs-CRP may add a benefit in determining the severity and monitoring of the control of bronchial asthma.

Introduction

The C-reactive protein (CRP) is an exquisitely sensitive non-specific marker of acute inflammation and tissue damage [1]. Standard assays for CRP lack the sensitivity needed to determine the levels of inflammation, and thus, recent improvements have resulted in a new generation of highly sensitive assays. CRP determined using a highly sensitive assay is referred to as high sensitivity-CRP (hs-CRP). Using hs-CRP, assessment of conditions indicative of chronic, low-grade inflammation is now possible. It has been demonstrated that hs-CRP is a strong independent predictor of future myocardial infarction, stroke, peripheral arterial disease and sudden cardiac death among healthy men and women, and recurrent events and death in patients with acute or stable coronary syndromes [2]. Also, low-level inflammation, as indicated by increased hs-CRP serum concentrations, has been described in both chronic obstructive pulmonary lung diseases (COPD) and asthma [3]. In asthma, not only local but also systemic inflammation occurs and hs-CRP may play a role in the pathogenesis of asthma [4].

Activated eosinophils play an important role in the pathogenesis of bronchial asthma. Eosinophils may contribute to airway hyper-responsiveness in asthma through the effects of eosinophil derived granular proteins on the bronchial epithelium [5]. Of these, is the eosinophilic cationic protein (ECP), which is largely responsible for the damage associated with eosinophil infiltration in bronchial mucosa [6].

Measuring of serum ECP levels has the advantages over eosinophil count as, it reflects not only the number of cells but also their degree of activation and therefore a better inflammatory marker [7]. Serum ECP levels are helpful in determining asthma activity and deciding the use of antiasthmatic drugs [8]. High serum ECP level may be a predictor and a risk factor for asthma exacerbation, thus it may have a useful role to play as a control parameter in asthma guideline [9].

The above mentioned data support the possible role of serum hs-CRP and ECP as inflammatory markers in bronchial asthma patients. However, the association between serum levels of hs-CRP and ECP and the degree of asthma control and the relationship between these levels and asthma severity have not been clearly identified yet. Hence, this study was carried out to investigate the association of serum levels of hs-CRP and ECP with the degree of asthma control, revealed by asthma control test (ACT), and to compare their levels among adults with mild, moderate and severe stable asthma.

Patients and methods

Patients

Thirty patients with, newly diagnosed, bronchial asthma (10 mild, 10 moderate and 10 severe) and 20 healthy non smoker controls were recruited randomly from the Pulmonary Outpatient Clinic between March 2013 and February 2014 in the Al-Adwani general hospital in Taif, Saudi Arabia. The diagnosis of asthma and the assessment of severity were performed according to the Global Initiative for Asthma (GINA) guidelines [10], supported by the presence of a clinical diagnosis of asthma (e.g. recurrent episodes of wheezing, breathlessness ,chest tightness, and coughing, particularly at night or in the early morning) and spirometric values showing reversibility of more than and/or equal to 12% in forced expiratory volume in 1 s (FEV_1), or at least 200 mL from baseline after inhalation of salbutamol (4×100 mcg) given by metered dose inhaler using a spacer device or peak expiratory flow (PEF) variability $\geqslant 20\%$.

Asthmatic patients were classified according to the *GINA* guidelines [10] (based on the level of symptoms, airflow limitation and lung function variability) into 3 subgroups: mild asthma (group I, $n = 10$); moderate asthma (group II, $n = 10$) and severe asthma (group III, $n = 10$).

A total of 15 asthmatic patients (5 out of each asthmatic subgroup i.e. 5 mild, 5 moderate and 5 severe) were followed up after 2 months of asthma therapy.

We excluded smokers, patients younger than 18 years, patients whose body mass index (BMI) was $\geqslant 25\ kg/m^2$, patients who had an exacerbation of asthma or respiratory tract infection during the last three months prior to enrollment and patients with hepatic; renal; cardiovascular diseases; diabetes mellitus; cancer or systemic inflammatory disorders. Written informed consents were obtained from all subjects.

Methods

All patients underwent a detailed symptom inquiry, physical examination and investigations including complete blood count (including circulating eosinophil (CE) count) , chest radiograph (postero-anterior view), pulmonary function test (PFT), serum total Immunoglobulin-E (IgE), serum hs-CRP, serum ECP and skin prick test (SPT). Also, assessment of asthma control test (ACT) and body mass index (BMI) was carried out for all subjects. Elevated serum total IgE level and positive SPT were used to define the atopic status.

PFT, ACT, CE and serum levels of total IgE, hs-CRP and ECP were reassessed for 15 asthmatic patients (5 out of each asthmatic subgroup) after 2 months of therapy to follow up the effectiveness of the treatment. Treatment of bronchial asthma was given according to the *GINA* guidelines [10].

Measurement of serum hs-CRP

Serum hs-CRP levels were measured using an available high sensitive-CRP commercial kit (ROCHE Diagnostics', COBAS C 501 auto analyzer, Germany). The measurement method used is based on a particle enhanced immunonephelometry

assay and quality control was done before the test measurement.

Measurement of serum ECP

Serum ECP levels were measured by the sandwich ELISA method using a commercially available test kit (MBL MESACUP ECP ELISA kit, Japan).

Measurement of serum total IgE

Serum total IgE levels were measured by the sandwich ELISA method using a commercially available test kit (cobas e 411 Germany).

Pulmonary function test

Spirometric PFT, with reversibility testing after inhalation of salbutamol (4×100 mcg) given by metered dose inhaler using a spacer device, was done using *Sensor Medicus 2450* computerized pulmonary function apparatus. Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) were measured. The best value of three maneuvers was expressed as a percentage of the predicted value and as absolute value.

Body mass index

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Skin prick test

Skin prick test was conducted with a panel of common aeroallergen extracts in the presence of a positive histamine control and a negative saline control on the forearm. Sixteen aeroallergens were utilized. The test was considered positive if the wheal was greater than 3 mm in mean diameter.

Asthma control test

ACT [11] was used to evaluate asthma control. The ACT questionnaire is a validated self-administered questionnaire including five questions related to the last four weeks: Episodes of breathlessness, nocturnal awakenings, limitations of daily activities, need for rescue medication and patient's self-rating of asthma control. Each question includes five response modalities with a score ranging from one to five by increasing level of asthma control, so the global arithmetic score ranges from five to twenty-five. Well controlled asthma by ACT was defined by a score $\geqslant 20$.

Statistical analysis

Statistical analysis was performed with the SPSS version 19 statistical software package (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SD or median and interquartile range (IQR). Student's *t* test was used for comparison between parametric data and Wilcoxon's rank sum test for non parametric data. Correlation analysis was carried out using Spearman's rank correlation test. *P* value < 0.05 was considered significant.

Results

Table 1 shows that serum ECP, serum hs-CRP, serum total IgE and CE were significantly higher ($p < 0.01$, 0.01, 0.05 and 0.05, respectively), while FEV_1 % and ACT were significantly lower ($p < 0.01$) in asthmatic patients compared to the control group.

Table 2 shows the comparative statistics for hs-CRP among different studied groups. Serum hs-CRP levels were significantly higher in all asthmatic patients, group I (mild asthma), group II (moderate asthma) and group III (severe asthma) compared to the control group ($p < 0.01$, 0.05, 0.01 and 0.01, respectively). In addition, serum hs-CRP levels were significantly higher in group III compared to group I and group II ($p < 0.01$) and in group II compared to group I ($p < 0.05$).

Table 3 shows the comparative statistics for serum ECP among different studied groups. Serum ECP levels were significantly higher in all asthmatic patients, group I, group II and group III compared to the control group ($p < 0.01$, 0.05, 0.05 and 0.01, respectively). Moreover, serum ECP levels were significantly higher in group III compared to group I and group II and in group II compared to group I ($p < 0.01$, respectively).

Table 4 shows correlation analysis of serum levels of hs-CRP and ECP against different studied parameters in all asthmatic patients. A significant positive correlation was noticed between serum levels of hs-CRP and ECP in asthmatic patients ($r_s = 0.67$, $p < 0.01$). Also, in all patients with bronchial asthma, serum levels of hs-CRP and ECP showed significant positive correlations with asthma severity (hs-CRP, $r_s = 0.59$, $p < 0.01$; ECP, $r_s = 0.63$, $p < 0.01$, respectively) but, significant negative correlations with ACT (hs-CRP, $r_s = -0.53$, $p < 0.05$; ECP, $r_s = -0.62$, $p < 0.01$, respectively) and FEV_1 % (hs-CRP, $r_s = -0.46$, $p < 0.05$; ECP, $r_s = -0.57$, $p < 0.01$, respectively). As regards CE, a significant positive correlation was seen with serum ECP ($r_s = 0.49$, $p < 0.05$) but, not with serum hs-CRP ($r_s = 0.39$, $p > 0.05$) in all asthmatic patients.

Table 5 shows descriptive and comparative statistics of all investigations in asthmatic patients (5 out of each asthmatic subgroup) before and after therapy. Serum ECP and hs-CRP levels showed a significant fall ($p < 0.01$and $p < 0.05$, respectively), while, FEV_1 % and ACT showed a significant increase ($p < 0.05$) but, CE and serum total IgE showed no significant change in the subgroup of asthmatic patients ($n = 15$) who were followed up after 2 months of asthma therapy.

Fig. 1 shows the receiver operating characteristic curve (ROC) applied to assess the diagnostic utility of ECP in discriminating moderate from severe asthma patients. The best diagnostic cutoff for ECP was 11.5 μg/L. This had a diagnostic sensitivity of 86.7%, specificity 60%, negative predictive value 60%, positive predictive value 86.7% and efficiency 80%. Area under the curve (AUC) was 0.682.

Discussion

Asthma is a widespread chronic disease. Its subjective and economic aspects require development of objective methods for the assessment of the disease activity, treatment efficacy, and, if possible, prevention of attacks [8]. For diagnostic and prognostic purposes some conventional markers are being

Table 1 Demographic, clinical and laboratory data among all studied population.

Parameter	Control group (n = 20) mean ± SD/median (IQR)	All asthmatics (n = 30) mean ± SD/median (IQR)	t (z^*)	p
Age (years)	32.6 ± 14.1	33 ± 12.5	0.12	>0.05
Sex: M/F	14/16	12/18	$X^2 = 0.08$[a]	>0.05
BMI (kg/m^2)	21 ± 2	22 ± 3	1.5	>0.05
FEV_1 % predicted	89.4 ± 9.6	69.5 ± 7.2	5.6	$p < 0.01$
ACT	25 ± 0	16 ± 4	4.2	$p < 0.01$
CE count (%)	4 (2–10)	11 (4–32)	2.2*	$p < 0.05$
Serum total IgE (IU/ml)	155 (89–273)	315 (152–425)	2.3*	$p < 0.05$
Serum hs-CRP (ng/L)	2.4 ± 1.7	16.3 ± 10	10.2	$p < 0.01$
Serum ECP (μg/L)	2.65 ± 0.92	17.7 ± 11.5	11.6	$p < 0.01$

[a] Chi square test is used; * Wilcoxon rank sum test is used; M/F, male/female; BMI, body mass index; FEV_1, forced expiratory volume in 1 s; ACT, asthma control test; CE, circulating eosinophil; hs-CRP, high sensitive C-reactive protein; ECP, eosinophilic cationic protein.

Table 2 Statistical comparison of serum hs-CRP among different studied groups.

Groups	z	p
Control vs. all asthmatic patients	−3.56	<0.01
Control vs. mild asthma patients	−2.42	<0.05
Control vs. moderate asthma patients	−2.95	<0.01
Control vs. severe asthma patients	−4.95	<0.01
Mild asthma patients vs. moderate asthma patients	−0.79	<0.05
Mild asthma patients vs. severe asthma patients	−3.62	<0.01
Moderate asthma patients vs. severe asthma patients	−3.08	<0.01

Hs-CRP, high sensitive C-reactive protein.

Table 3 Statistical comparison of serum ECP among different studied groups.

Groups	z	p
Control vs. all asthmatic patients	−3.9	<0.01
Control vs. mild asthma patients	−2.97	<0.05
Control vs. moderate asthma patients	−1.9	<0.05
Control vs. severe asthma patients	−4.19	<0.01
Mild asthma patients vs. moderate asthma patients	−0.53	<0.01
Mild asthma patients vs. severe asthma patients	−2.77	<0.01
Moderate asthma patients vs. severe asthma patients	−2.86	<0.01

ECP, eosinophilic cationic protein.

used by the physicians and investigators for the past years like FEV_1 % predicted, CE count, serum IgE level etc. Now, it has been suggested that eosinophil may contribute to airway hyperresponsiveness in asthma through the effects of eosinophil derived granular proteins on the bronchial epithelium such as ECP, major basic protein (MBP), eosinophil peroxidase (EPO) and eosinophil derivative neurotoxin (EDN) [12].

In asthma, the importance of airway inflammation has been well established. Besides the airway inflammation, systemic inflammation may also exist in asthma. The relevance of high sensitivity assays for hs-CRP, which is known to be a sensitive marker of low-grade systemic inflammation, has not been fully studied in asthma. Studies have attempted to validate the use of hs-CRP as a surrogate marker of airway inflammation in bronchial asthma [13].

The present work aimed to study the clinical utility of biological markers hs-CRP and ECP for severity classification of asthma and follow up therapy.

The results of this study showed that serum ECP, serum hs-CRP, serum total IgE and CE were significantly higher ($p < 0.01$,0.01, 0.05 and 0.05, respectively), while FEV_1 % and ACT were significantly lower ($p < 0.01$) in asthmatic patients compared to the control group. Our results agreed with those of Zedan et al. [6], Takemura et al. [13] and Kilic et al. [14]. Such results illustrate the importance of airway inflammation in asthma and support the hypothesis that beside the airway inflammation, systemic inflammation may also exist in patients with bronchial asthma.

Our study revealed that serum hs-CRP levels were significantly higher in all asthmatic subgroups; group I (mild asthma); group II (moderate asthma) and group III (severe asthma) compared to the control group ($p < 0.05$, 0.01 and 0.01, respectively). In addition, serum hs-CRP levels were significantly higher in group III compared to group I and group II ($p < 0.01$) and in group II compared to group I ($p < 0.05$). Also, serum hs-CRP showed significant positive correlation with asthma severity and significant negative correlation with ACT and FEV_1 % of predicted in all studied asthmatic patients. These results are in accordance with Takemura et al. [13], Kilic et al. [14] and Qian et al. [15] who explained that inflammatory process differs with staging of asthma after the exclusion of the role of infection. The severity of the disease significantly relies on the volume of the bronchial inflammation and the amount of inflammatory mediators released in circulation and hence the acute phase reactant will be synthesized by the liver, CRP is one of the most important acute phase reactants.

The relationship between the levels of hs-CRP and the severity of asthma indicates that CRP is a proinflammatory agent [14]. Different proinflammatory cytokines such as interleukin-1β (IL-1β), IL-6, IL-8 and IL-18 have been synthesized by the activated protein hs-CRP. These cytokines are increased due to asthmatic inflammation [16,17]. Severity of asthma correlates positively with asthmatic inflammation [18].

Our study demonstrated that serum ECP levels were significantly higher in all asthmatic subgroups; group I; group II and group III compared to the control group ($p < 0.05$, 0.05 and 0.01, respectively). Moreover, serum ECP levels were

Table 4 Correlation analysis of serum levels of hs-CRP and ECP against different studied parameters in all asthmatic patients ($n = 30$).

Parameter	Serum hs-CRP (ng/L)		Serum ECP (μg/L)	
	r_s	p	r_s	p
Asthma severity	0.59	< 0.01	0.63	< 0.01
CE count (%)	0.39	> 0.05	0.49	< 0.05
ACT	−0.53	< 0.05	−0.62	< 0.01
FEV_1 % predicted	−0.46	< 0.05	−0.57	< 0.01
Serum hs-CRP (ng/L)	.....	.....	0.67	< 0.01

FEV_1, forced expiratory volume in 1 s; ACT, asthma control test; CE: circulating eosinophil; hs-CRP, high sensitive C-reactive protein; ECP, eosinophilic cationic protein.

Table 5 Descriptive and comparative statistics of all investigations in asthmatic patients before and after therapy.

Parameter	Before therapy ($n = 15$) mean ± SD/median (IQR)	After therapy ($n = 15$) mean ± SD/median (IQR)	p
FEV_1 % predicted	72 ± 7	81 ± 8.6	$p < 0.05$
ACT	17 ± 3	20 ± 4	$p < 0.05$
CE count (%)	12 (1–22)	11 (2–19)	$p > 0.05$
Serum total IgE (IU/ml)	155 (56–221)	135 (44–198)	$p > 0.05$
Serum hs-CRP (ng/L)	18.9 ± 6.8	12.4 ± 5.4	$p < 0.05$
Serum ECP (μg/L)	22.4 ± 8.7	4.9 ± 2.3	$p < 0.01$

FEV_1, forced expiratory volume in 1 s; ACT, asthma control test; CE: circulating eosinophil; hs-CRP, high sensitive C-reactive protein; ECP, eosinophilic cationic protein.

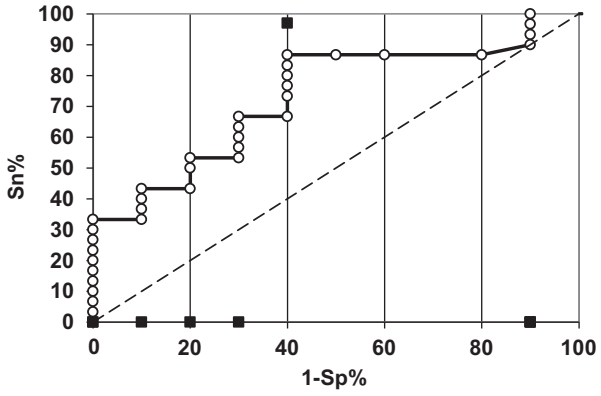

Figure 1 ROC curve analysis showing the diagnostic performance of serum ECP for discriminating moderate asthma from severe asthma.

significantly higher in group III compared to group I and group II and in group II compared to group I ($p < 0.01$, respectively). Also, serum ECP showed significant positive correlation with asthma severity and CE but, significant negative correlation with ACT and FEV_1 % of predicted in all studied asthmatic patients. These results are in accordance with Hoshino and Nakamura [19], Koh et al. [20] and Badar et al. [21].

Zedan et al. [6] and Begum et al. [22] explained that serum ECP is now accepted as an indicator of eosinophil activity. It appears to be the earliest indicator of allergen induced bronchial inflammation when changes in symptoms or in bronchial responsiveness are not yet evident.

In accordance with the previous reports [8,22], the current work showed that serum ECP levels had significantly fallen ($p < 0.01$), while, FEV_1 % and ACT had significantly increased ($p < 0.05$) in the subgroup of asthmatic patients who were followed up after 2 months of asthma therapy. Begum et al. [22] explained that elevated ECP seems to denote patients at the risk of inflammatory exacerbations, resulting in practical implications for the therapeutic management of chronic asthma. ECP is a guide to tail down inhaled corticosteroid therapy and assessment of compliance to most forms of anti-inflammatory therapies in asthma and guiding the tapering of inhaled corticosteroid in stabilized asthmatics. Spirometric measurements (FEV_1) may not accurately reflect the extent of the inflammatory process in the airways and thereby do not always indicate the best choice of monitoring therapy.

Moreover a strong positive correlation was found between ECP and hs-CRP in all asthmatic patients. This supports the hypothesis that both are inflammatory markers that could increase in any inflammatory condition such as asthma, but ECP is more specific for asthma than hs-CRP.

Assessment of the diagnostic performance of ECP revealed that the best cutoff value for discriminating patients with moderate from patients with severe asthma was 11.5 μg/L. At this value, serum ECP had a diagnostic sensitivity of 86.7%, specificity of 60%, negative predictive value of 60%, positive predictive value of 86.7% and efficiency of 80%. This result needs further validation by larger wide scale future studies.

To our knowledge, this is one of the first reports trying to elucidate the effect of therapy on serum hs-CRP levels in patients with bronchial asthma. We noticed that serum hs-CRP levels had significantly fallen ($p < 0.05$) in the subgroup

of asthmatic patients who were followed up after 2 months of asthma therapy. Only one previous study by Takemura et al. [13] did not find a significant difference in serum hs-CRP levels between their asthmatic patients who were on inhaled corticosteroids (ICS) and healthy controls and reported that the ICS, which has well characterized anti-inflammatory properties, used in these patients might have reduced serum hs-CRP.

In conclusion, coupling measurements of serum levels of both ECP and hs-CRP may add a benefit in determining the severity of asthma and hence, early addressing the step of therapy. Furthermore, objective monitoring of asthma control using simple easy to measure serum markers could be achieved. Serum ECP could effectively discriminate moderate from severe asthmatic patients.

Further study

Establishment of a reference range for serum levels of ECP and hs-CRP in asthmatic patients and cutoff values for different disease stages are needed.

Conflict of interest

None.

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